

# **Report for 2003TN7B: Evaluation of Pathogen Occurrence and Causation withing the Stock Creek Watershed (Knox County) as a Model for Watershed Restoration**

- Articles in Refereed Scientific Journals:
  - Gentry, R W; J McCarthy, A Layton, L McKay, G S Sayler, Fecal Bacteria Occurrence at Basal Conditions in a Karst Dominated Watershed in East Tennessee, (in preparation).
  - Layton, A D; V Williams, L Garrett, R McKay, R Gentry, G. Sayler, Development of Bacteroides 16srRNA gene TaqMan PCR assays for the quantification of fecal source pollution in water samples, Submitted to Applied and Environmental Microbiology in 2004, (currently in revision).
- Other Publications:
  - Gentry, R W; J McCarthy, A Layton, L McKay, S Koirala, 2004, Evaluating microbial water quality and potential sources of contamination in a small rural watershed in karstic terrain, Groundwater Quality 2004-4th International Conference, July 19th - 22nd, 2004, University of Waterloo, Ontario, Canada.
  - Gentry, R W; 2004, Assessing Ground Water and Surface Water Interaction through Tracer Observation, ASCE-EWRI, 2004 World Water & Environmental Resources Congress, June 27 July 1, 2004, Salt Lake City, Utah.
  - Gentry, R W; J McCarthy, A Layton, L McKay, S Koirala, 2004, A Hydrologic Investigation into the Occurrence and Causation of Pathogen Indicators in the Stock Creek Watershed, Knoxville, Tennessee, TN Section American Water Resources Association, Thirteenth Annual Tennessee Water Resources Symposium, March 31 April 2, 2004, Burns, TN.
  - Gentry, R W; J McCarthy, A Layton, L McKay, S Koirala, 2004, A hydrologic investigation into the occurrence and causation of pathogen indicators in the Stock Creek watershed, Knoxville, TN, AWRA 14th Annual Tennessee Water Resources Symposium, Burns, TN, March 31-April 2, 2004.
  - Layton, A; D Williams, V Garrett, L McKay, R Gentry, J McCarthy, G Sayler, 2004, Development of real-time PCR assays for the detection of Bacteroides sp. as a method to quantify fecal contamination, TN Section American Water Resources Association, Thirteenth Annual Tennessee Water Resources Symposium, March 31 April 2, 2004, Burns, TN.
  - Layton, A; L McKay, D Williams, V Garrett, R Gentry, J McCarthy, G Sayler, 2004, Development of real-time PCR assays for the detection of Bacteroides sp., KY/TN Water Environment Association, Pollutant Source Identification Specialty Conference, Murfreesboro, TN, May 20, 2004.
  - Layton, A; D Williams, V Garrett, L McKay, 2004, Development of real-time PCR assays for the detection of Bacteroides sp. as a method to quantify fecal contamination, AWRA 14th Annual Tennessee Water Resources Symposium, Burns, TN, March 31-April 2, 2004.
  - McKay, L; A Layton, T Baldwin, G Sayler, 2004, Development and testing of real-time PCR assays for detecting pathogens and related microbial indicators in karst settings, GQ2004 Conference, Waterloo, Ontario, Canada, July 19-22, 2004.

Report Follows

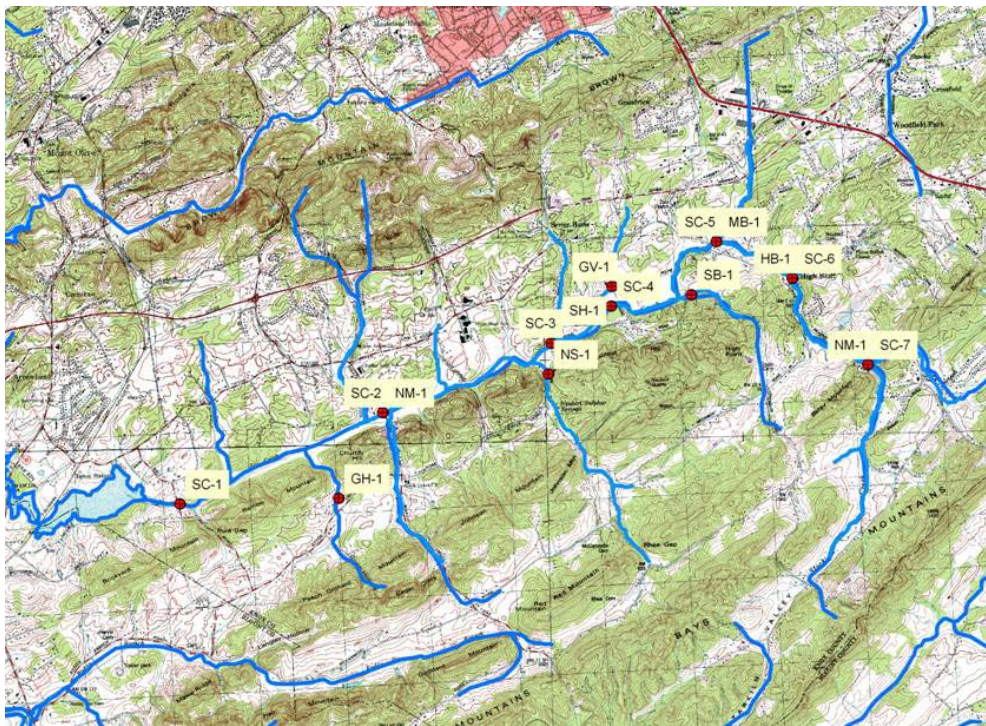
**Problem and Research Objective:**

This proposal focuses on the Stock Creek Watershed, which is part of the Little River Watershed in Knox and Blount Counties. Stock Creek, which is on the 303(d) list because of elevated levels of pathogens, represents a small watershed that can serve as a “research watershed” to explore and evaluate strategies for holistic approaches to watershed remediation. The principal goal is an evaluation of pathogen occurrence and causation within the creek that will eventually lead to the restoration of the Stock Creek Watershed. The project is testing and demonstrating holistic watershed assessment protocols that can serve as a model for restoration of other impaired waterbodies. We are testing innovative monitoring techniques to distinguish between human- and animal sources of pathogens, and evaluate the effectiveness of integrated stream assessment tools in watershed restoration. The project will achieve a secondary goal of using the process knowledge of the watershed to restore it to the condition fully supporting its designated uses, and lead to the removal of Stock Creek from the 303(d) list before a TMDL is required.

This project objectives are to (1) devise and implement a monitoring plan to obtain information on water quality (water chemistry, pathogens, sediment load, biological integrity), (2) further develop biomolecular tools that will provide real-time data on levels of pathogen indicators and of pathogenic *E. coli* and that will directly help to (3) distinguish between human- and animal-derived pathogens, and potential “pathogen reservoirs” which could be persistent sources of pathogen inputs to the Creek, and (4) the role of storm events, seasonal changes in hydrologic conditions, and groundwater inputs to water quality and pathogen levels. The new fundamental knowledge of pollutant sources and watershed processes can be used by partnering agencies to develop recommendations for BMPs, and support efforts to obtain Grant Pool funds to perform the BMPs. The success of the integrated watershed remediation and management tools developed within this project can be demonstrated by the restoration of the Stock Creek Watershed to its designated uses, and its removal Stock Creek from the 303(d) list.

**Methodology and Accomplishments to Date:**

The Stock Creek Watershed has been evaluated based on sampling from 16 locations that were selected to monitor water quality at several locations within the stream, as well as at locations where major tributaries draining sub-watersheds enter Stock Creek. We obtained data on water quality, stream flow rate and pathogens at the sampling locations at approximate monthly intervals for a year. The sampling locations are shown in Figure 1. The data are being used in several ways.



**Figure 1.** Stock Creek and associated sample locations.

Data are being made available in Access format to use by TDEC or for incorporation into GIS layers for use by other agencies such as TVA and Knoxville-Knox County to assist in identifying potential pollution sources. The types of data that have been collected include categories of pathogens, water quality, and hydrology.

Pathogen indicators have been quantified at each of the sampling locations using “standard” analyses for total coliforms and fecal coliforms by the TDEC laboratory in Knoxville. In addition, two alternative methods have been compared. The EPA-approved Hack pathogen test kit involves filtration of water samples and incubation of the filters. Dyes in the kit identify lactose utilizing and glucuronidase-containing organisms to provide information on levels of total coliforms and *E. coli*. We have developed real-time PCR assays for the differentiation and quantification of fecal anaerobic bacteria within the genus *Bacteroides*. This research was performed in conjunction with two other projects monitoring the Stock Creek Watershed. Data collected in this research project is expected to provide information regarding the sources of fecal contamination (cattle versus human) necessary for the development of a TMDL for pathogens in the Stock Creek Watershed.

Water chemistry and hydrology of Stock Creek is being analyzed to evaluate the sources of the pathogens and to help design effective BMPs. Water chemistry of the water samples has been analyzed by TDEC. Both base flow and storm monitoring of the Creek provides information to parameterize a hydrologic flow model of the watershed. This effort helps link pathogen loading to storm events and to sediment resuspension processes. An important aspect of this work will also include an analysis of overland flow travel times. The evaluation will attempt to correlate the concentration of pathogens to the time of concentration of sub-basin catchments.

**Principal Findings and Significance:**

1. In conjunction with two other Stock Creek projects, a real-time PCR assay was developed to differentiate and quantify fecal anaerobic bacteria within the genus *Bacteroides*.
2. It appears that bovine-specific *Bacteroides* are present throughout the Stock Creek Watershed and are at high levels at some sites. This suggests that a portion of the *E. coli* contamination in Stock Creek may be attributable to cattle. Additional data analysis is needed to determine if there is a link between the levels of bovine-specific *Bacteroides* and *E. coli*
3. Frequency analysis was performed on several main channel sites. The analysis provided a probabilistic model for the occurrence of general and bovine specific fecal loading within the watershed. of This is a potentially valuable statistical approach for risk-based modeling and establishment of regulatory limits within the TMDL process. The methodology may also be useful for post-BMP audits for evaluating BMP performance in varying watershed types.

**Future Research and Funding:**

A hydrologic model of the watershed is being developed based on flow monitoring of the Creek. These efforts will continue as part of Shesh Koirala's Ph.D. research in the Department of Civil and Environmental Engineering.